

Realtime Chemical Bioimaging: Direct Detection of Chemicals (O_2) in Biological Samples

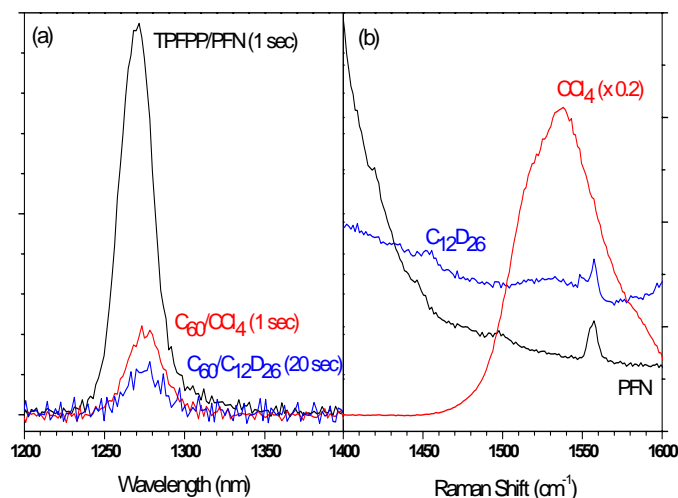
NIST is developing a coherent anti-Stokes Raman scattering (CARS) spectroscopy/microscopy system for direct chemical imaging in biological systems. In particular, the new system will be capable of detecting “minority” chemical species, such as reactive O , an important molecule involved in bacterial killing and tissue damage associated with reperfusion injury after heart attacks and stroke. The new technology is expected to enable new innovations in drug discovery research and understanding of the molecular pathology of human disease.

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Imaging modalities in biological systems generally can be divided into two classifications: “whole body” – where contrast is sought in macro-scale systems (i.e., organs to organisms) and “cellular” – where contrast at micrometer (and less) length scales are used to probe specific regions within cellular structures. While fluorescence based bioimaging has provided a wealth of valuable information with respect to biosystems, there is a great need to develop cellular level *chemical* imaging, where extrinsic contrast agents (i.e., dyes and stains) are not needed and more specific detail can be provided with respect to inherent/intrinsic chemical functional groups.

In recent years, coherent anti-Stokes Raman scattering (CARS) has emerged as an innovative chemical imaging technique for biosystems, with sensitivities in some cases (typically “majority” species – e.g., CH_x in lipids) allowing for video rate (i.e., real time) imaging. The primary goals of this exploratory research project were to 1) develop a CARS spectroscopy/microscopy system, and 2) evaluate the use of CARS in the detection of “minority” species, specifically focusing on O_2 , in biological systems. Ultimately, if favorable characteristic detection sensitivities are apparent, this CARS capability would be directed towards the measurement of singlet O_2 [$O_2(^1\Delta)$], which is an unstable, highly reactive species that is critical in photodynamic therapy.

Direct, real-time visualization of chemical, such as reactive oxygen, in biological samples, without the use of probes, is being made possible.



The NIR emission of three PS/solvent systems is shown in (a) with respective integration times indicating the dynamic range of the observed ($^1\Delta$) O_2 concentrations. Raman spectra of the neat solvents in (b) show the extent of interferences; the CCl_4 spectrum has been multiplied by 0.2 and the peak at 1555 cm^{-1} in both PFN and $C_{12}D_{26}$ is dissolved oxygen.

Over the last decade, two primary configurations have been developed for CARS bioimaging, with each having attributes that (somewhat) favor different applications. The most “typical” CARS configuration uses two picosecond lasers, and this is well-suited for applications targeting signal maximization from a vibrational resonance at a specific frequency. With this, a single channel detector can be used and video rate, species-specific imaging is feasible. Alternatively, broadband CARS can be performed using a femtosecond and a picosecond laser. This scheme allows for the acquisition of a CARS spectrum for each pulse pair (i.e., “shot”), with the spectral coverage determined by the bandwidth of the femtosecond pulse (can approach 2000 cm^{-1}). Further, for both schemes, there are a number of experimental conditions that allow for significant performance enhancements, with one of the most advantageous being the use of well-defined and optimal polarization states for input and output beams, so called P-CARS (Polarization-CARS). In P-CARS, the polarization conditions can be fixed to quite effectively eliminate large detrimental nonresonant signals, which can be orders of magnitude greater than targeted resonant signals for weak/dilute vibrational features. Our work thus far has focused on P-CARS using two picosecond pulses, with spectra over a small frequency interval (several 10 's cm^{-1}) obtained via

wavelength scanning one of the lasers. We have obtained P-CARS spectra of minor solvent bands, and are currently working towards the detection of dissolved O_2 , which based on spontaneous Raman results has an intensity that is substantially smaller (i.e., $> \times 10$) than indicated solvent features.

Concurrent with our development of a P-CARS system, we have determined a sample solution for the production of $O_2(^1\Delta)$ that will serve as testbed for the feasibility of CARS detection of this species. $O_2(^1\Delta)$ can be effectively formed from ground state $O_2(^3\Sigma)$ via interaction with a photosensitizer (PS), and the $O_2(^1\Delta)$ concentration is proportional to the $^1\Delta \rightarrow ^3\Sigma$ emission intensity at 1270 nm (NIR). A number of PS/solvents systems were evaluated with the primary criteria being 1) insignificant solvent vibrational features determined via spontaneous Raman at about 1480 cm^{-1} and 1555 cm^{-1} to minimize interferences with $O_2(^1\Delta)$ and $O_2(^3\Sigma)$ and 2) maximize steady state concentration of $^1\Delta$ determined from NIR emission.

It is known that O_2 has high solubility in nonpolar solvents, and deuterated/fluorinated solvents provide minimal spectral interferences and typically afford long emission lifetimes for $O_2(^1\Delta)$. It was found that tetra(perfluoropropyl) porphyrin (TPFPP)/perfluorononane (PFN) gave the highest NIR emission. In general, C_{60} /perdeuteroalkanes provided about $\times 100$ less $^1\Delta$ NIR emission than the TPFPP/PFN system and emission for Methylene Blue/ D_2O was below the detection limit.

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Future Plans: The NIST team will continue to optimize the performance of the CARS configuration towards the development of both video rate imaging of majority species as well as a probe of the spatial distribution of minority species in biosystems. This capability will be extended to address metrology needs in materials science/reliability, including the determination of stress fields with micrometer resolution.